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Brief Research Communication

Autism-Related Routines and Rituals Associated With a Mitochondrial Aspartate/Glutamate Carrier SLC25A12 Polymorphism

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Evidence for a genetic association between autism and two single nucleotide polymorphisms (SNPs), rs2056202 and rs2292813, in the mitochondrial aspartate/glutamate carrier (SLC25A12) gene led us to ask whether any of the four previously identified familial traits in autism spectrum disorders (ASD) varied by these SNPs. In 355 ASD cases from 170 sibships we examined levels of the four traits in these SNPs using ANCOVA models. The primary models selected unrelated affected cases and used age and sex as covariates. An ancillary set of models used *all* affected siblings and included "sibship" as a random effects independent variable. We found significantly lower levels of routines and rituals associated with the presence of the less frequent A allele in rs2056206. No other significant differences were observed. The rs2056202 polymorphism may be associated with levels of routines and rituals in autism and related disorders.

KEY WORDS: repetitive behaviors; familial traits; autism spectrum disorders

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INTRODUCTION

Evidence for a genetic association with autism was recently reported for two single nucleotide polymorphisms (SNPs) in the mitochondrial aspartate/glutamate carrier gene (SLC25A12) [Ramoz et al., 2004]. While negative studies have been reported [Blasi et al., 2006; Correia et al., 2006; Rabionet et al., 2006], these results have been replicated with the same SNPs in an independent sample and another group reported a significant association with one of these SNPs [Segurado et al., 2005; Turunen et al., 2007]. Other previous work suggests that the age when phrase speech first appears, the level of language attained, and two higher order repetitive behavior traits—"preoccupations/circumscribed interests" and "compulsive adherence to nonfunctional routine and rituals"—are familial traits in autism and related disorders [Silverman et al., 2002]. Here, these four traits were examined in relation to the two SLC25A12 polymorphisms in a large series of multiply affected siblings with autism or related disorders.

METHODS

Ascertainment of Families and Diagnostic Classification

The current sample were those families included in our earlier genetic association study [Ramoz et al., 2004] that were assessed either by our center alone or in conjunction with the Autism Genetic Resource Exchange (AGRE). The ascertainment and diagnostic methods have been previously described [Silverman et al., 2002]. Briefly, with parents providing written informed consent, we recruited families with at least one member meeting ICD-10/DSM-IV autism and at least a second with either autism or autism-related disorder. Trained, reliable interviewers administered the Autism Diagnostic Interview—Revised (ADI-R) [Lord et al., 1994] with the primary caregiver for each suspected case. An autism diagnosis via the ADI-R depends on the onset age of symptoms and scores above thresholds on each of the three autism domains (i.e., social interaction, communication, and repetitive behaviors). Domain scores are the sums of "category" scores (e.g., "routines and rituals" (D2) is one of the four repetitive behavior categories). To classify participants not meeting autism criteria on the ADI-R, we used a previously described diagnostic hierarchy including Asperger disorder, borderline autism, autism spectrum disorder (ASD), or autism-related developmental deficits (ARDD) [Silverman et al., 2002].

Genotyping

As described previously [Ramoz et al., 2004], polymerase chain reaction (PCR) was used to genotype two G/A intronic SNPs, rs2056202 and rs2292813, in the SLC25A12 gene. Many other SNPs in the 2q24-q33 region were examined earlier, but only these two gave evidence for association with autism [Ramoz et al., 2004; Segurado et al., 2005].

Statistics

We used analysis of covariance to examine the four familial traits by genotype first to directly examine the effects of the SNP polymorphisms after controlling for age and sex in

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unrelated affected cases. These covariates were included because each was likely to be associated with the traits under investigation. Because multiple-affected siblings were not independent observations, in our primary analyses we selected one case from each sibship. In sibships where the less common A allele was present, we selected the member carrying that allele or, if there were more than one, chose one using a randomizing procedure. In families where affected siblings were concordant for the G/G genotype we selected one sibling using the same randomizing procedure. A second series of analyses included *all* affected siblings, age and sex as covariates, as well as the random effects independent variable, "sibship." These latter models allowed us to examine, for each trait, whether the SNP polymorphisms act as within-sibship effect modifiers.

RESULTS

There were 355 cases assessed (mean age: 8 ± 5 years; male/female: 3.6/1) from 170 multiply affected sibships. The less common A allele for rs2292813 had a low frequency (8.6%), was present in only 37 families, and only 16 sibships had discordant genotypes-limiting statistical power to detect differences in the four familial traits for this SNP and none were observed. For rs2056202, 284 subjects were G/G homozygotes, 68 subjects were G/A heterozyotes, and 3 were A/A homozygotes. Affected cases with the latter two genotypes came from 50 unrelated families and we randomly selected one of them for the initial set of analyses. For this SNP there were 35 families with discordant genotypes among affected cases. Fifteen of the 170 sibships had three affected cases and 155 had two. There were 291 autism cases and 64 with autism-related disorders: Aspergers: n = 15; borderline autism: n = 17; ASD: n = 14; ARDD: n = 18. There were no differences in the distribution of G/G homozygotes and the 1+ A allele cases group for autism ($\chi^2 = 0.58$, df = 1, n.s.) or the specific autism-related disorder classifications ($\chi^2 = 4.65$, df = 4, n.s.).

The Table I shows the estimated marginal mean values, after covarying for age and sex, for each familial trait in those carrying at least one A allele versus those with no A allele (G/G genotypes). The routines and rituals category was significantly lower in those with 1+ A allele. There were no other significant differences for the remaining three familial traits; however, level of language was more impaired at a trend level among the 1+ A allele group. As there was a significant negative correlation between routines and rituals and level of language ($r = -0.29$, $P < 0.001$), in an ancillary model, we included level of language as an additional covariate with no other changes and found that the significant group difference with respect to the routines and rituals category remained ($F[1,165] = 4.53$, $P < 0.05$). Conversely, including the routines and rituals category as a covariate in the model with level of language as the dependent variable substantially reduced the earlier trend ($P = 0.25$).

In a secondary series of analyses we used all family members and included "sibship" as a random effects variable. Again, G/G homozygotes had higher routines and rituals scores than those with 1+ A alleles ($F(1, 36.3) = 5.03$, $P < 0.05$). The results did not change when level of language was included as a covariate ($F[1,35.5] = 4.55$, $P < 0.05$). Independent within-sibship analyses of the other three familial traits were not significant (all P-values > 0.35).

We plotted the residualized scores for routines and rituals (i.e., after covarying by age and sex) in the 35 sibships (Fig. 1) that had discordant genotypes between the affected cases to visually examine within-family relationships for rs2056202. The upward shift of the lowest points across the x-axis is an artifact of the ordering method (by low scoring sibling). However, the observable, albeit not wholly consistent, tendency for those carrying an A allele to have lower routines and rituals scores than their own G/G siblings reflects the overall statistical finding for rs2056202 with this trait. Similarly, in the one G/A–A/A sibship (#23), the heterozygote's score was higher than the homozygote's.

DISCUSSION

Controlling for age and sex, a significant difference in the routines and rituals category was observed among the cases of autism and autism-related disorders according to rs2056202 genotype, one of two SNPs in the AGC1 gene with replicated, albeit not uniformly consistent, evidence indicating a genetic association with autism [Ramoz et al., 2004; Segurado et al., 2005; Blasi et al., 2006; Correia et al., 2006; Rabionet et al., 2006; Turunen et al., 2007]. This was true both in a comparison using unrelated (i.e., independent) affected cases as well as using the full dataset with "sibship" in the model. While all the subjects had an autism-related disorder, those carrying the A allele had significantly lower routines and rituals score indicative of less impairment. As our own and other groups found that this minor allele was positively associated with greater protection from autism overall [Ramoz et al., 2004; Segurado et al., 2005; Turunen et al., 2007], the present results may provide an indication of a specific familial autism trait that may be attenuated when this allele is present.

The association between rs2056202 A allele and less severe routines and rituals might perhaps be explained by the trend level correlation between level of language and the rs2056202 SNP, especially as one of the items, "verbal rituals," included in the routines and rituals score requires that useful phrase speech be present. However, including the level of language score as a covariate, did not meaningfully affect the rs2056202 association with routines and rituals. On the other hand, the trend level effect with level of language, a borderline result that might be attributable to reduced power due to its restricted scale (three points), was in any case reduced substantially when the routines and ritual category was entered as a covariate. Age at phrase speech, which was used by our group and others to identify the locus on chromosome 2 [A genome-wide screen for autism, 2001; Buxbaum et al., 2001; Shao et al., 2002], was not associated with the rs2056202 genotype. This characteristic, however, was not employed in the studies of SLC25A12 and autism and the earlier evidence for genetic

TABLE I. ADI-R Familial Trait Scores in One Affected Subject Per Family* Grouped by the Presence or Absence of At Least One A Allele for rs2056202

rs2056205	Age (months) at phrase speech	Level of language	Circumscribed interests	Routines and rituals
1+ A allele (n = 50)	45 (2)	0.90 (0.12)	1.31 (0.11)	0.79 (0.17)
G/G (n = 120)	44 (4)	0.63 (0.08)	1.54 (1.21)	1.30 (0.11)
Statistics[a]	$F(1,166) = 0.05$, $P = 0.83$	$F(1,166) = 3.25$, $P = 0.07$	$F(1,166) = 1.36$, $P = 0.25$	$F(1,166) = 6.49$, $P = 0.0117$

*Due to the low frequency of the A allele, we randomly selected among affected siblings carrying one or more A alleles when the affected siblings were not all G/G genotypes. When all affected siblings had the same genotype one was randomly selected.
[a]The F statistic represents the test of the presence or absence of at least one A allele after covarying for age and sex.

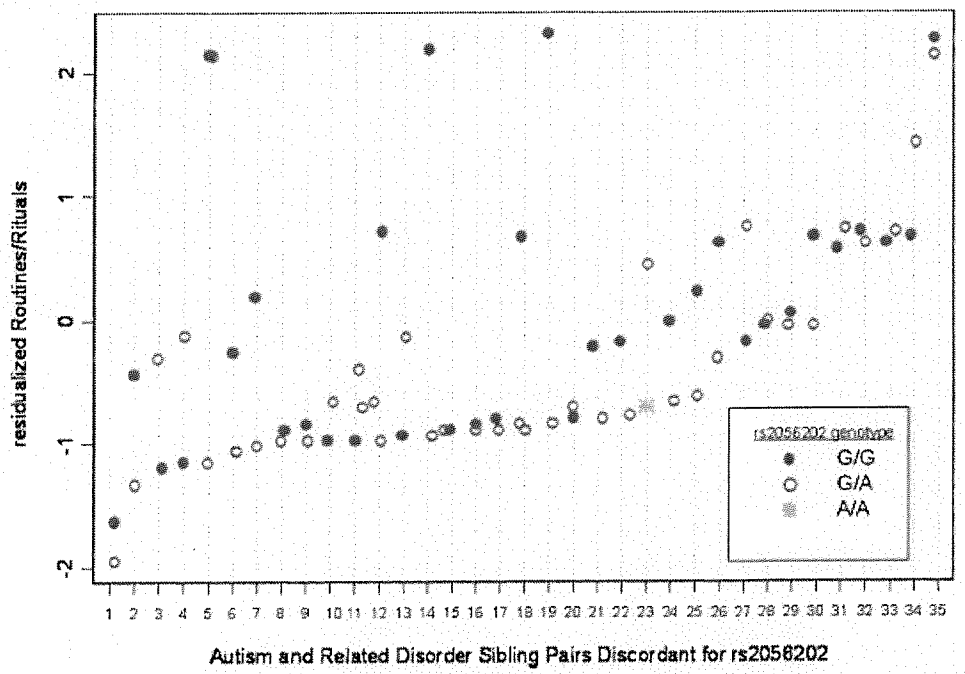

Fig. 1. Residualized (by age and sex) ADI-R routines and ritual scores (y axis) in autism and related disorder cases grouped by membership to multiply affected sibships (x axis) that are discordant for the rs2056202 polymorphism in the SLC25A12 gene. Symbols for G/G homozygotes are filled circles, G/A heterozygotes are open circles, and the sole A/A homozygote by an open square. The sibships are ordered left to right according to the low scoring member within each sibship. The coordinates for x axis (sibship) have been slightly jittered to reveal scoring that may be overlapping. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

association did not depend on this characteristic [Ramoz et al., 2004; Segurado et al., 2005; Turunen et al., 2007]. Finally, the preoccupations and circumscribed interests category along with all the other ADI-R categories (including deficits in communicative gestures, which previously was observed as familial in autism alone, but not in the broader autism-related disorders [Silverman et al., 2002]), investigated in ancillary analyses, were not associated with rs2056202.

These findings require replication in independent samples. However, they suggest that, in addition to the evidence for an association with autism in general, the rs2056202 polymorphism in the SLC25A12 gene may help explain variability of routines and rituals within autism and related disorders. As other genes associated with this and other autism-related characteristics are identified [Sakurai et al., 2006], it will be interesting to examine the joint effects on this and related intermediate phenotypes.

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